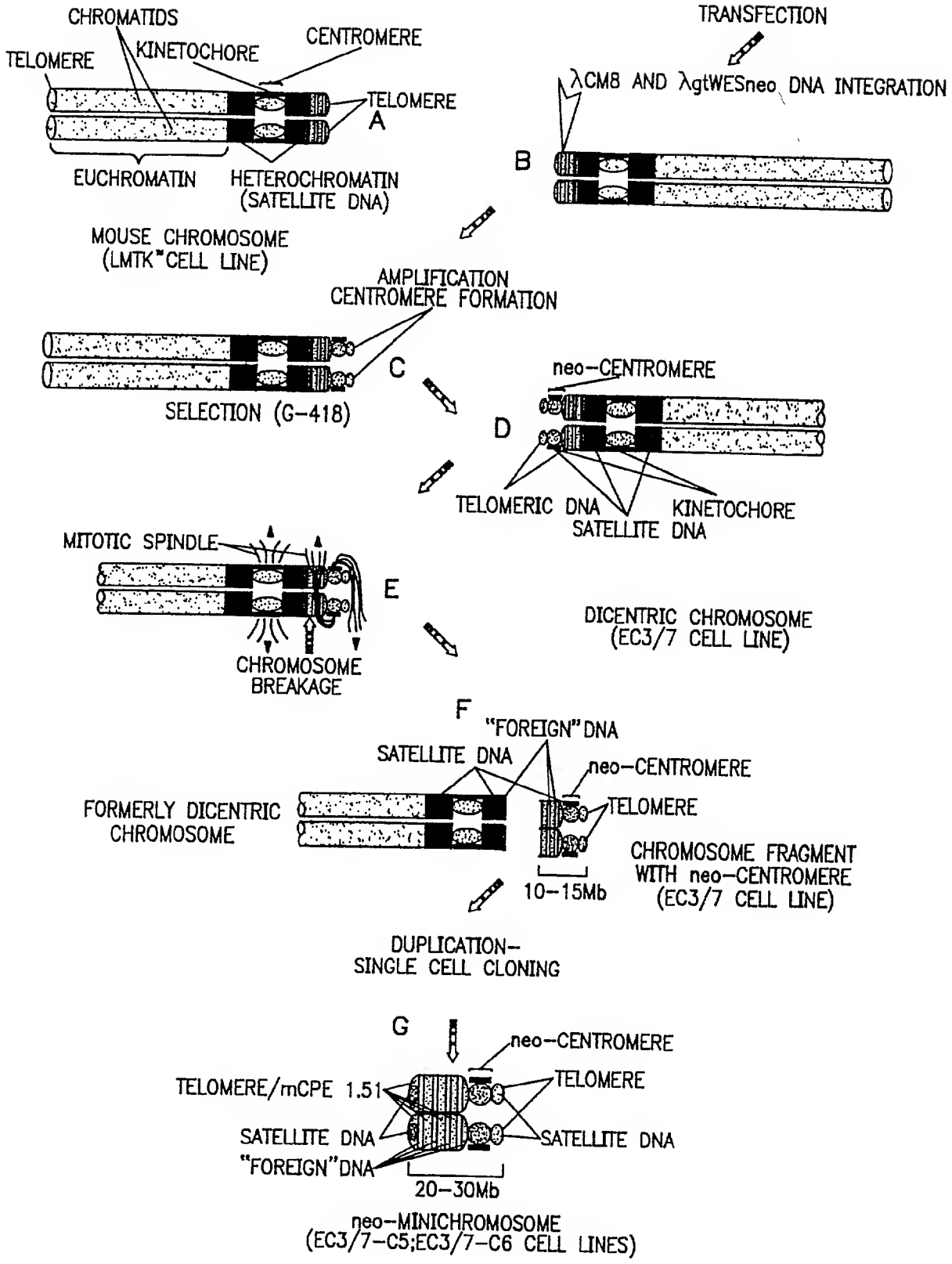

CHROMATIDS
TELOMERE
KINETOCHORE
CENTROMERE
TELOMERE
A
EUCHROMATIN
HETEROCHROMATIN
(SATELLITE DNA)
MOUSE CHROMOSOME
(LMTK⁻CELL LINE)
TRANSFECTION
λCM8 AND λgtWESneo DNA INTEGRATION
B
AMPLIFICATION
CENTROMERE FORMATION
C
SELECTION (G-418)
neo-CENTROMERE
D
TELOMERIC DNA
KINETOCHORE
SATELLITE DNA
MITOTIC SPINDLE
E
CHROMOSOME
BREAKAGE
DICENTRIC CHROMOSOME
(EC3/7 CELL LINE)
F
"FOREIGN" DNA
SATELLITE DNA
neo-CENTROMERE
FORMERLY DICENTRIC
CHROMOSOME
TELOMERE
10-15Mb
CHROMOSOME FRAGMENT
WITH neo-CENTROMERE
(EC3/7 CELL LINE)
DUPLICATION-
SINGLE CELL CLONING
G
neo-CENTROMERE
TELOMERE/mCPE 1.51
TELOMERE
SATELLITE DNA
SATELLITE DNA
"FOREIGN"DNA
20-30Mb
neo-MINICHROMOSOME
(EC3/7-C5;EC3/7-C6 CELL LINES)

FIG. 1

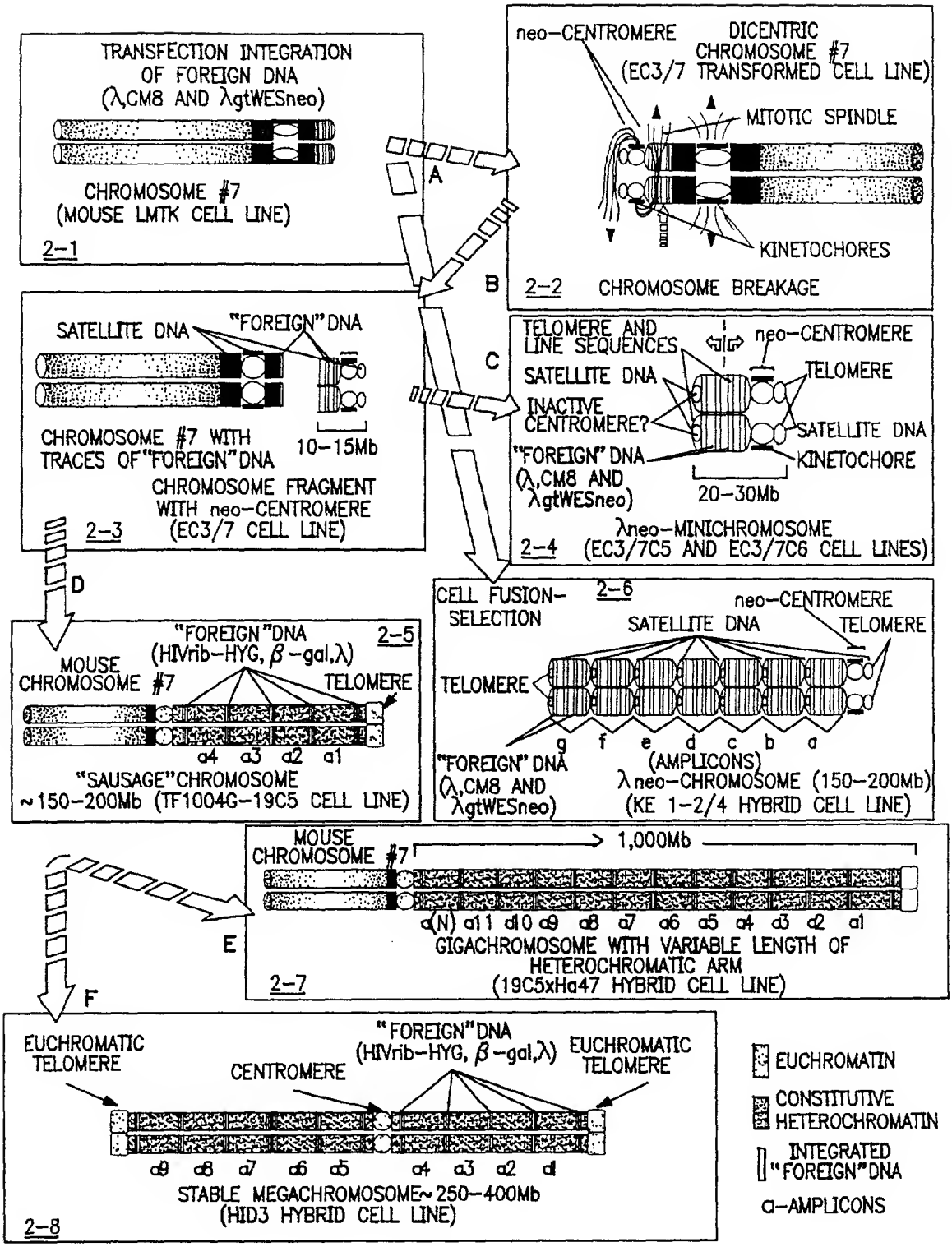

TRANSFECTION INTEGRATION OF FOREIGN DNA (λ,CM8 AND λgtWESneo)
CHROMOSOME #7 (MOUSE LMTK CELL LINE)
2-1
A
neo-CENTROMERE
DICENTRIC CHROMOSOME #7 (EC3/7 TRANSFORMED CELL LINE)
MITOTIC SPINDLE
KINETOCHORES
B
2-2 CHROMOSOME BREAKAGE
SATELLITE DNA
"FOREIGN" DNA
CHROMOSOME #7 WITH TRACES OF "FOREIGN" DNA
10-15Mb
CHROMOSOME FRAGMENT WITH neo-CENTROMERE (EC3/7 CELL LINE)
2-3
C
TELOMERE AND LINE SEQUENCES
neo-CENTROMERE
SATELLITE DNA
TELOMERE
INACTIVE CENTROMERE?
SATELLITE DNA
"FOREIGN" DNA (λ,CM8 AND λgtWESneo)
KINETOCHORE
20-30Mb
λneo-MINICHROMOSOME (EC3/7C5 AND EC3/7C6 CELL LINES)
2-4
D
"FOREIGN" DNA (HIVrib-HYG, β-gal,λ)
2-5
MOUSE CHROMOSOME #7
TELOMERE
α4 α3 α2 α1
"SAUSAGE" CHROMOSOME ~150-200Mb (TF1004G-19C5 CELL LINE)
CELL FUSION-SELECTION
2-6
neo-CENTROMERE
SATELLITE DNA
TELOMERE
TELOMERE
g f e d c b a
"FOREIGN" DNA (λ,CM8 AND λgtWESneo)
(AMPLICONS)
λneo-CHROMOSOME (150-200Mb) (KE 1-2/4 HYBRID CELL LINE)
E
MOUSE CHROMOSOME #7
1,000Mb
α(N) α11 α10 α9 α8 α7 α6 α5 α4 α3 α2 α1
GIGACHROMOSOME WITH VARIABLE LENGTH OF HETEROCHROMATIC ARM (19C5xHa47 HYBRID CELL LINE)
2-7
F
EUCHROMATIC TELOMERE
CENTROMERE
"FOREIGN" DNA (HIVrib-HYG, β-gal,λ)
EUCHROMATIC TELOMERE
α9 α8 α7 α6 α5 α4 α3 α2 α1
STABLE MEGACHROMOSOME~250-400Mb (HID3 HYBRID CELL LINE)
2-8
EUCHROMATIN
CONSTITUTIVE HETEROCHROMATIN
INTEGRATED "FOREIGN" DNA
α-AMPLICONS

FIG. 2

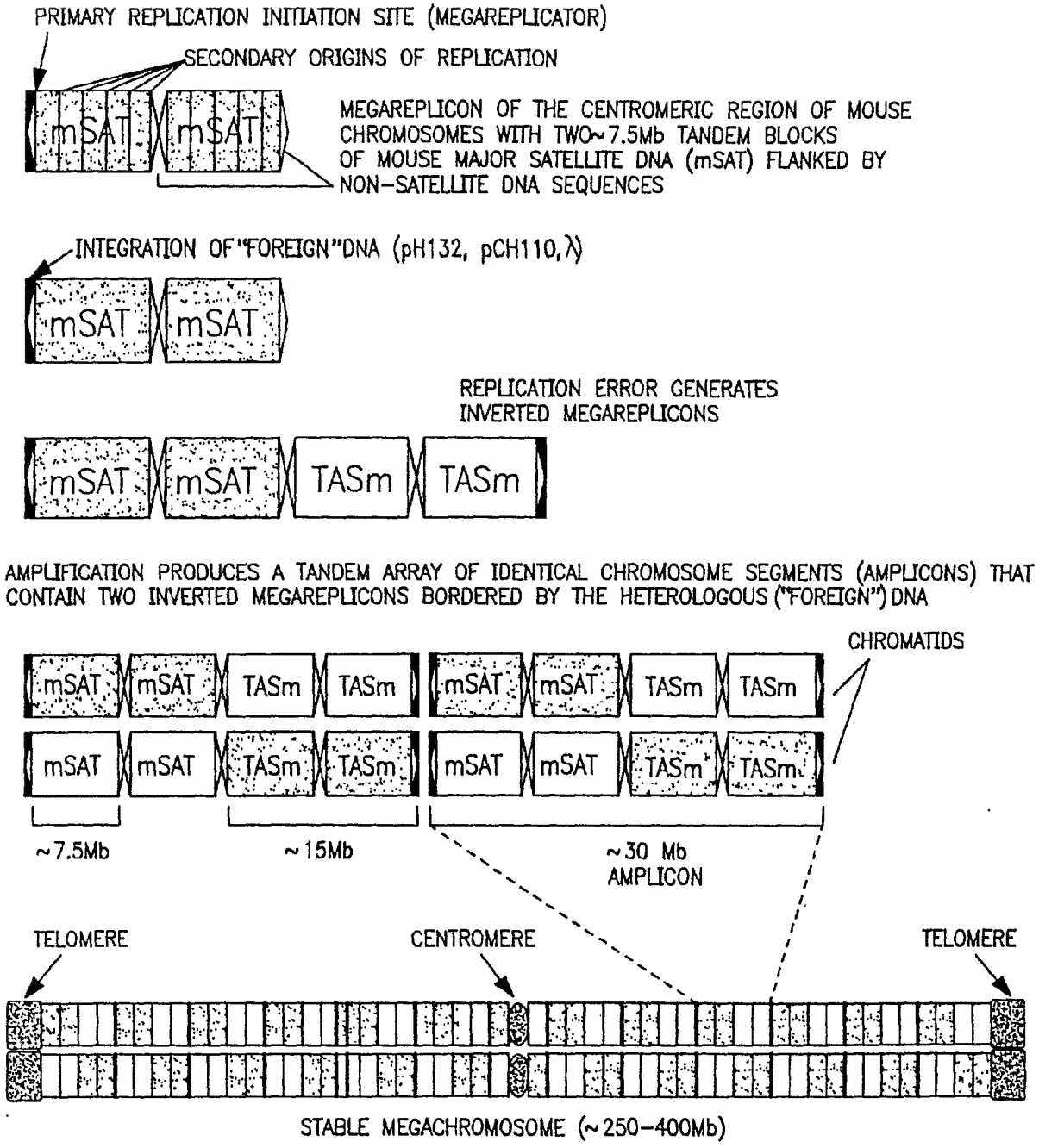

PRIMARY REPLICATION INITIATION SITE (MEGAREPLICATOR)
SECONDARY ORIGINS OF REPLICATION
mSAT
mSAT
MEGAREPLICON OF THE CENTROMERIC REGION OF MOUSE CHROMOSOMES WITH TWO~7.5Mb TANDEM BLOCKS OF MOUSE MAJOR SATELLITE DNA (mSAT) FLANKED BY NON-SATELLITE DNA SEQUENCES
INTEGRATION OF "FOREIGN" DNA (pH132, pCH110, λ)
mSAT
mSAT
REPLICATION ERROR GENERATES INVERTED MEGAREPLICONS
mSAT
mSAT
TASm
TASm
AMPLIFICATION PRODUCES A TANDEM ARRAY OF IDENTICAL CHROMOSOME SEGMENTS (AMPLICONS) THAT CONTAIN TWO INVERTED MEGAREPLICONS BORDERED BY THE HETEROLOGOUS ("FOREIGN") DNA
CHROMATIDS
~7.5Mb
~15Mb
~30 Mb AMPLICON
TELOMERE
CENTROMERE
TELOMERE
STABLE MEGACHROMOSOME (~250-400Mb)

FIG. 3

EC3/7 MOUSE LMTK FIBROBLAST CELL LINE WITH neo-CENTROMERE (HADLACZKY ET AL. PROC. NATL. ACAD. SCI. USA, 88: 8106-8110, 1991)

↓ DEPOSITED IN THE EUROPEAN COLLECTION OF ANIMAL CELL CULTURE (ECACC) ACCESSION NUMBER 9005 1001

↓ SINGLE-CELL SUBCLONING

EC3/7CSMOUSE LMTK FIBROBLAST CELL LINES WITH neo-MINICHROMOSOME (HADLACZKY ET AL. PROC. NATL. ACAD. SCI. USA, 88: 8106-8110, 1991)

↓ COTRANSFECTION WITH PLASMIDS pH132 (HIVRIBOZYME, HYGROMYCIN RESISTANCE) pCH110 (β-GALACTOSIDASE), AND LAMBDA PHAGE (λC1 875 SAM7) DNA, SELECTION WITH HYGROMYCIN B.

TF1004G-19C5* - MOUSE LMTK FIBROBLAST CELL LINES WITH neo-MINICHROMOSOME, AND STABLE "SAUSAGE" CHROMOSOME

↓ FUSION WITH CHINESE HAMSTER (CHO K20) CELL LINE, SELECTION WITH HYGROMYCIN B AND HAT.

19C5xHα4 - MOUSE-HAMSTER HYBRID CELL LINE CARRYING THE neo-MINICHROMOSOME AND THE "SAUSAGE" CHROMOSOME, CONTAINING COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME.

↓ BrdU TREATMENT, SINGLE CELL CLONING, SELECTION: G418 (NEOMYCIN) OR HYGROMYCIN, OR BOTH

G3DS*- MOUSE-HAMSTER HYBRID CELL LINE CARRYING THE neo-MINICHROMOSOME AND THE MEGACHROMOSOME, CONTAINING COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME.

↓

H1D3*- MOUSE-HAMSTER HYBRID CELL LINE CARRYING NO neo-MINICHROMOSOME BUT THE MEGACHROMOSOME, IS PRESENT, CONTAINING COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME.

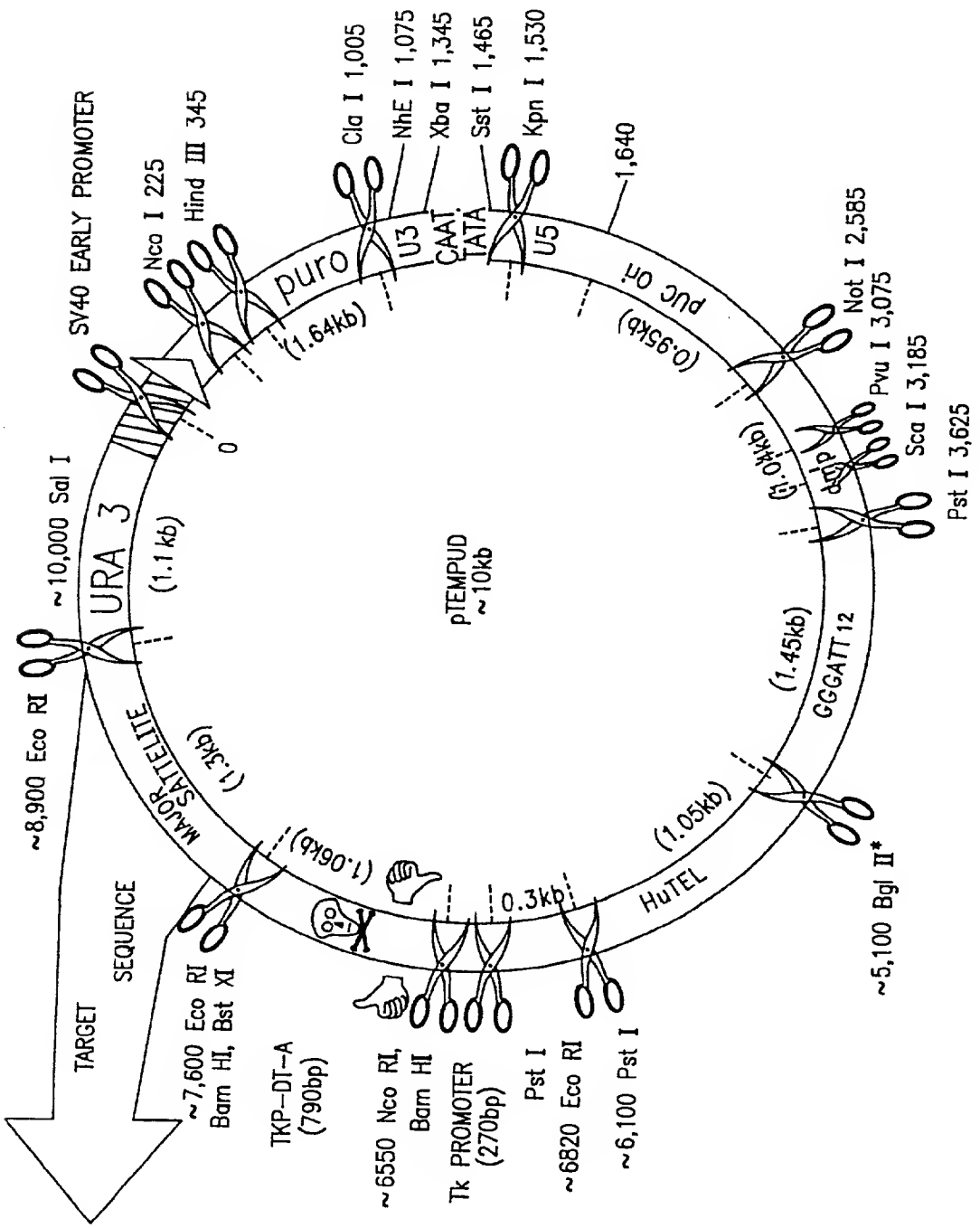
↓ FUSION WITH CD4+ HeLa CELL LINE CARRYING THE CD4 AND NEOMYCIN RESISTANCE GENE PLASMID CONSTRUCT (CD4neo), SELECTION WITH G418 AND HYGROMYCIN B

H1xHe41*- MOUSE-HAMSTER-HUMAN HYBRID CELL LINE CARRYING THE MEGACHROMOSOME PRESENT, CONTAINING COMPLETE HAMSTER GENOME, AND PARTIAL MOUSE GENOME, AND A SINGLE HUMAN CHROMOSOME WITH INTEGRATED CD4neo CONSTRUCT (UNPUBLISHED).

↓ REPEATED BrdU TREATMENT, SINGLE-CELL CLONING

1B3 - SAME AS H1xHe41, BUT APPROXIMATELY 25% OF THE CELLS ARE CARRYING A TRUNCATED MEGACHROMOSOME

Figure 4


pTEMPUD
~10kb
SV40 EARLY PROMOTER
Nco I 225
Hind III 345
puro
(1.64kb)
Cla I 1,005
NhE I 1,075
Xba I 1,345
Sst I 1,465
Kpn I 1,530
1,640
U3
CAAT
TATA
U5
puc ori
(0.95kb)
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Pst I
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Bam HI
Tk PROMOTER
(270bp)
TKP-DT-A
(790bp)
~7,600 Eco RI
Bam HI, Bst XI
(1.06kb)
(1.3kb)
MAJOR SATELITE
~8,900 Eco RI
~10,000 Sal I
URA 3
(1.1 kb)
0
TARGET
SEQUENCE

FIG. 5